

Scheme 1 Solid Phase Protein Synthesis
Native Chemical Ligations in an N- to C- Terminal Direction

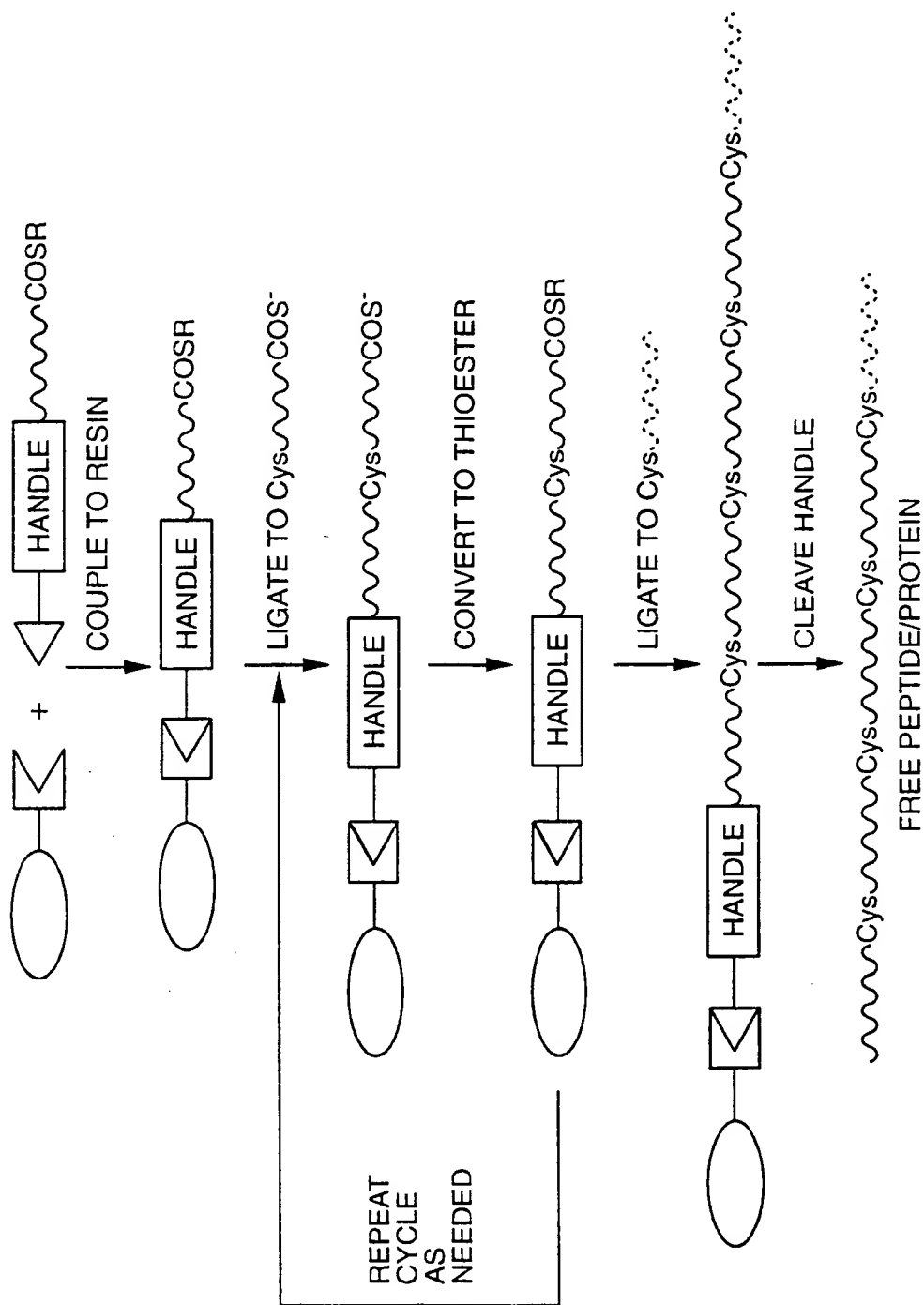
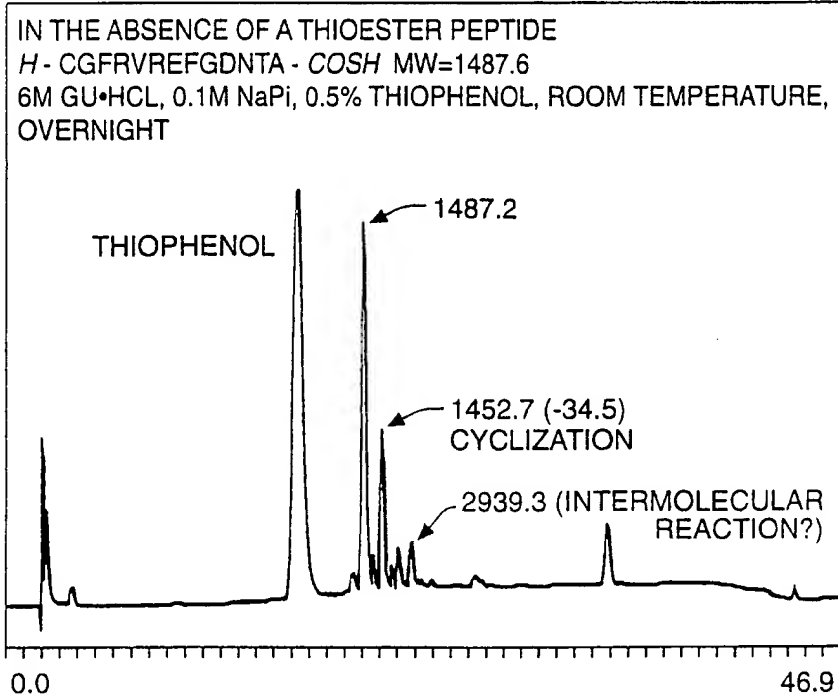
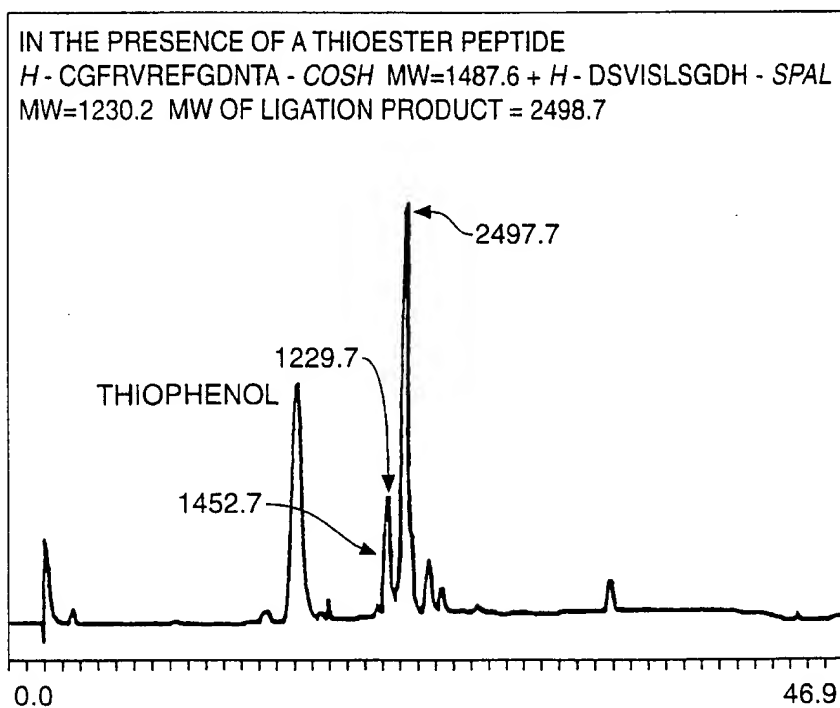
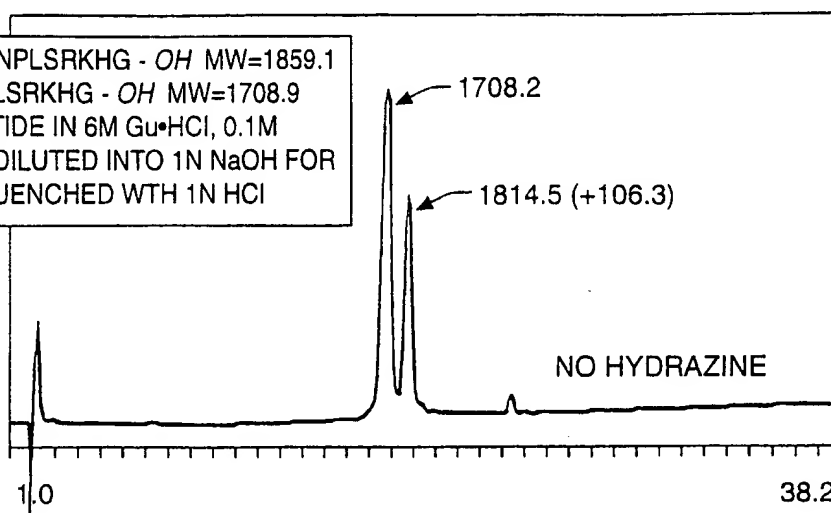


FIG. 1

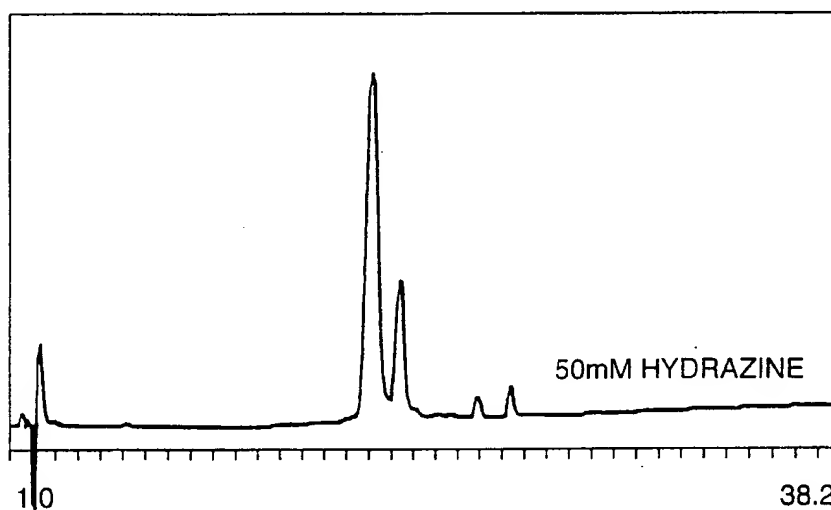
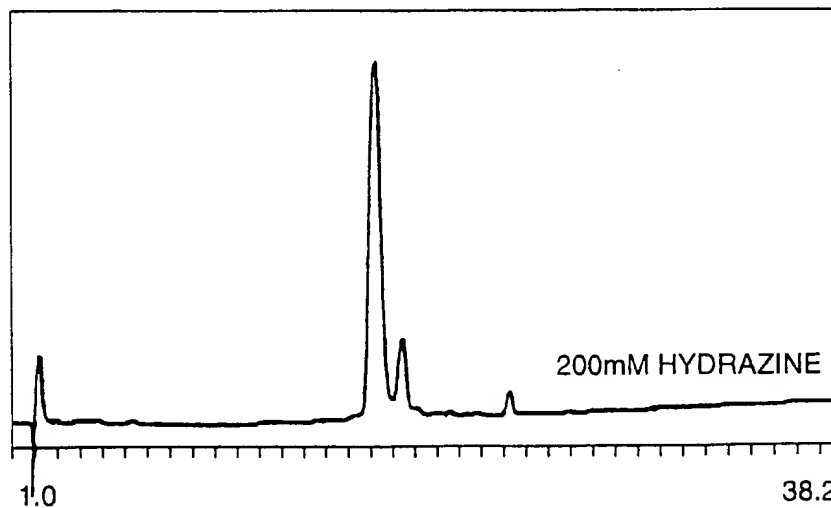
**FIG._2A****FIG._2B**

Cys +COSR Stability Under Ligation Conditions

MSC - CTSAGPHFNPLSRKHG - OH MW=1859.1
H - CTSAGPHFNPLSRKHG - OH MW=1708.9
ALIQUOT OF PEPTIDE IN 6M GU•HCl, 0.1M
NaPi, pH 7.5 WAS DILUTED INTO 1N NaOH FOR
TWO MINUTES, QUENCHED WITH 1N HCl

**FIG._3A**

MSC
Removal
Experiments

**FIG._3B****FIG._3C**

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH
 MW=4022.4
H - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH
 MW=3745.1
 ALIQUOT OF PEPTIDE IN 6M Gu•HCl, 0.1M NaAc, pH 4.6 WAS DILUTED
 INTO 6M Gu•HCl, 0.1M NaAc, pH 14 FOR TWO MINUTES, QUENCHED WITH
 6M Gu•HCl, 0.1M NaAc, pH2.0

MSC Removal
 Experiments
 (Cont'd)

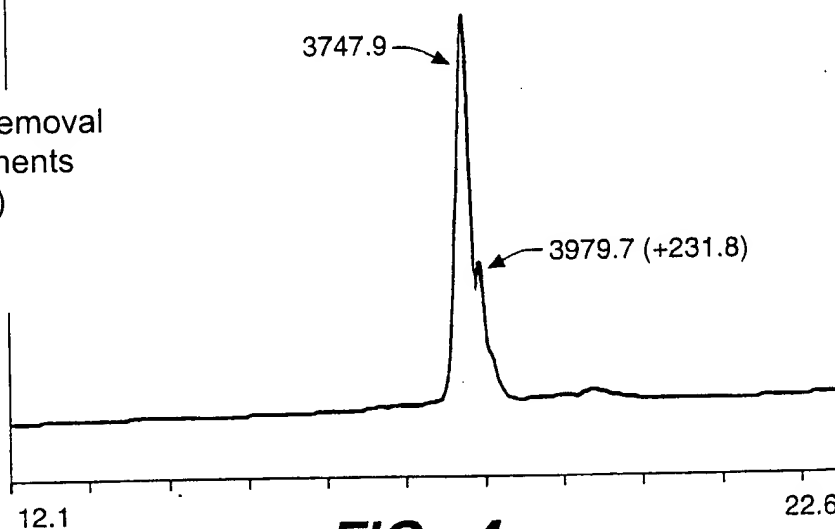


FIG. 4

1 21 47
 TLQKKIEEIAAKYKHSVVKKCCYDGACVNNDETCEQRAARISLGPKCIKAFTECC
 VVASQLRANISHKDMQLGR
 74

Synthesis of C5a by Solid
 Phase Chemical Ligations
 in the N- to C-Terminal
 Direction

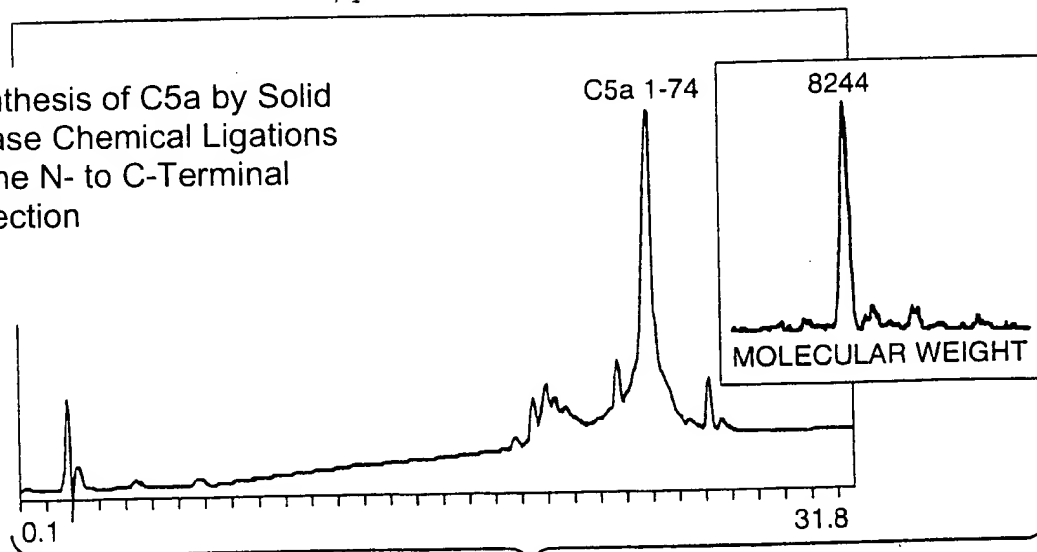
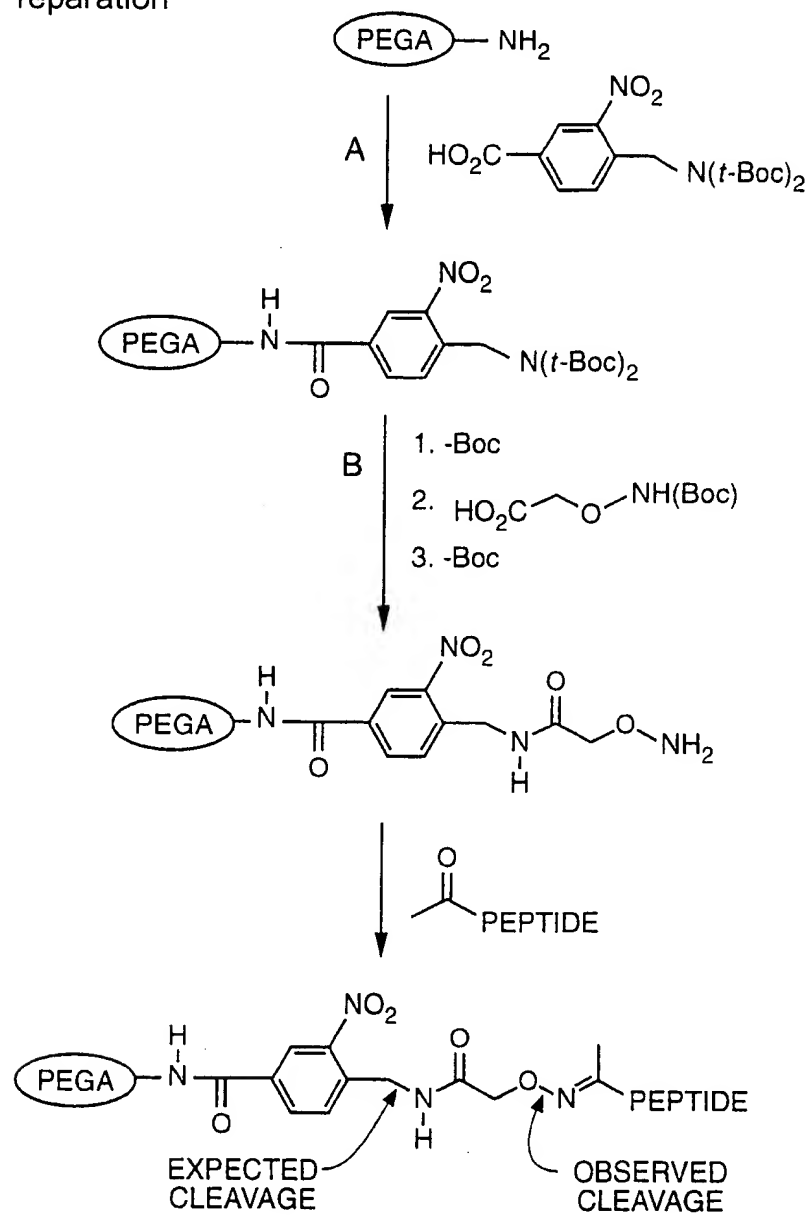
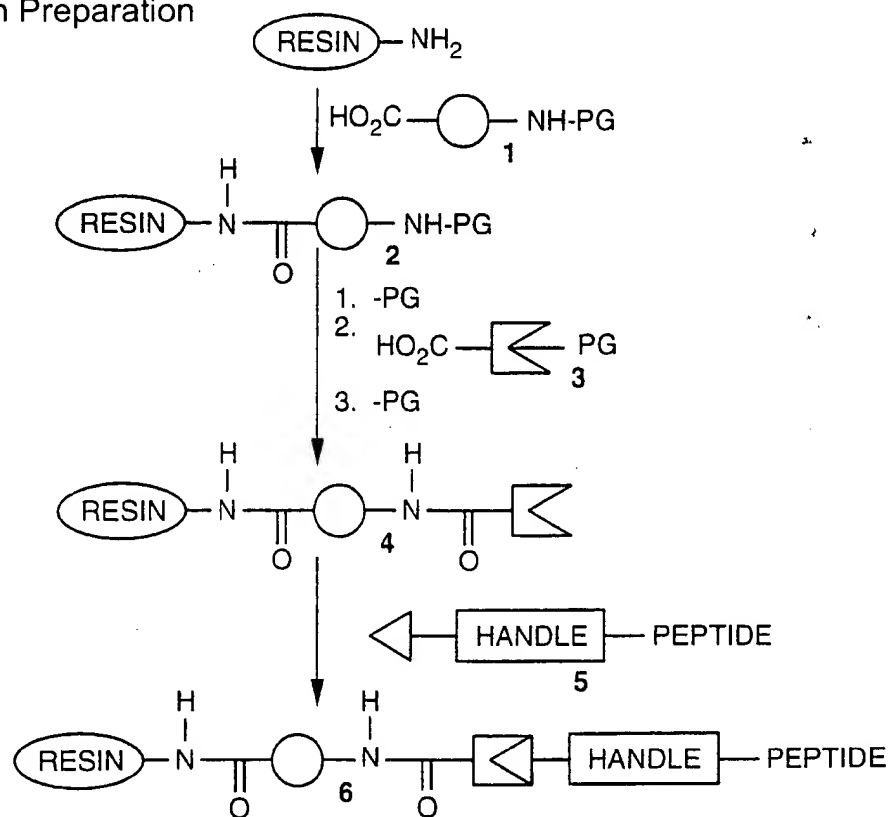


FIG. 26

Resin Preparation

**FIG. 5A**

Resin Preparation



$\text{HO}_2\text{C}-\text{circle}-\text{NH}-$ = CLEAVABLE LINKER USED FOR MONITORING WITH MALDI, ELECTROSPRAY MASS SPECT, ETC...

PG = PROTECTING GROUP

$\text{HO}_2\text{C}-\triangle-$ = FUNCTIONAL GROUP ADDED TO RESIN TO COUPLE WITH PEPTIDE

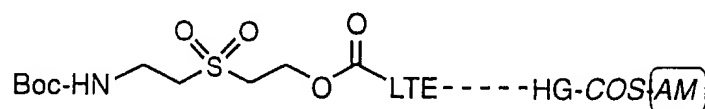
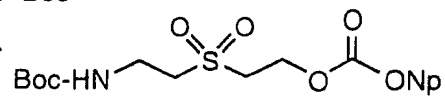
$\triangle-\text{HANDLE}-\text{PEPTIDE}$ = PEPTIDE FUNCTIONALIZED WITH
1. CLEAVABLE HANDLE FOR RELEASE OF PEPTIDE/PROTEIN FROM THE RESIN AT COMPLETION OF SYNTHESIS AND
2. FUNCTIONAL GROUP TO COUPLE TO RESIN

FIG. 5B



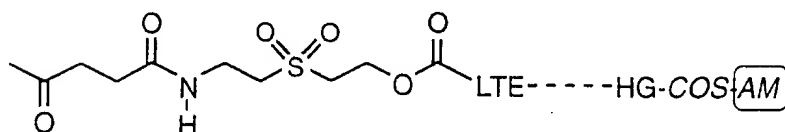
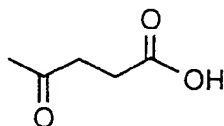
1. -Boc

2.



1. -Boc

2.



HF CLEAVAGE

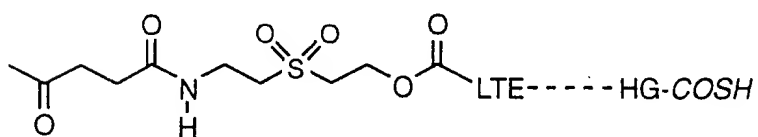


FIG. 6

Derivatization of Segment 1
(N-terminal)

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH(1)
+ Resin - PCL - ONH₂

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH(1)

Polymer-Supported Ligation on PEGA

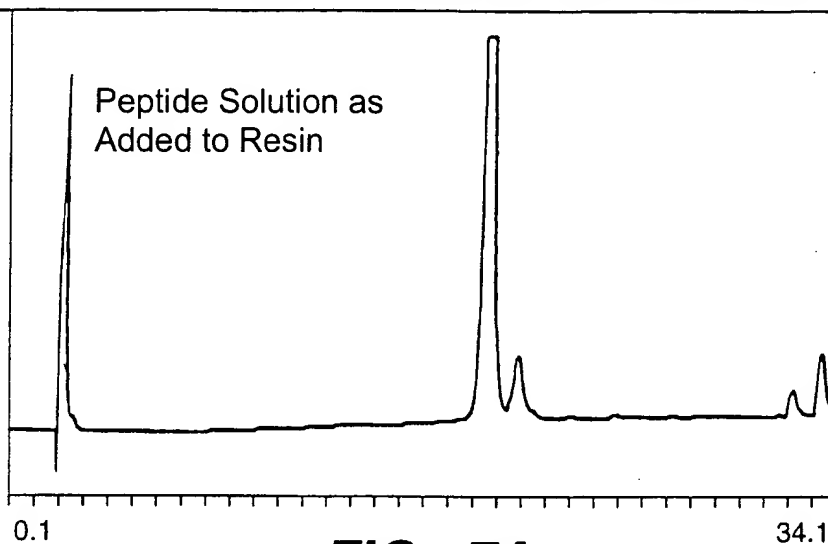


FIG. 7A

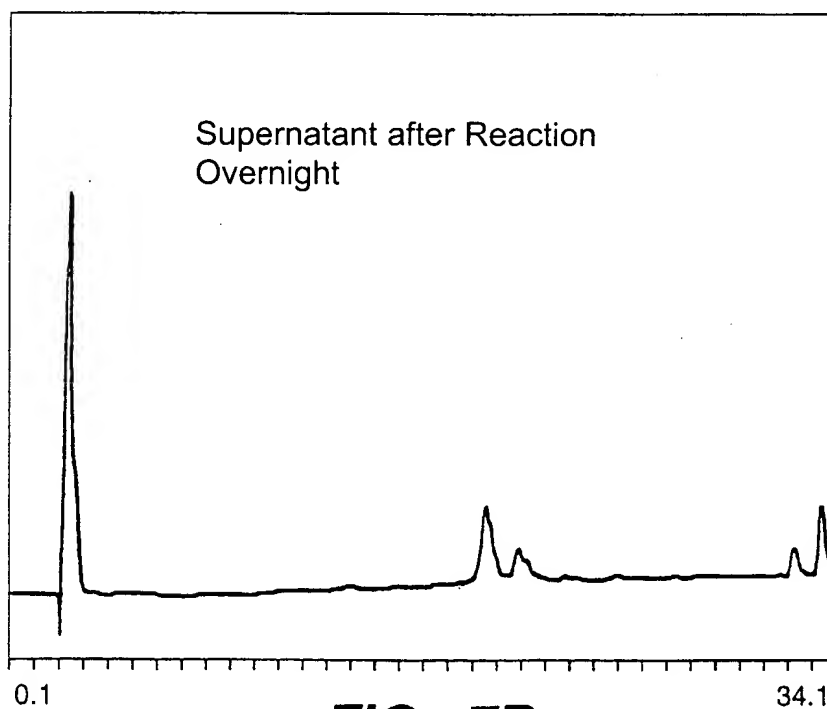


FIG. 7B

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH(1)
+ Resin - PCL - ONH₂

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH(1)

Polymer-Supported Ligation on ISCO

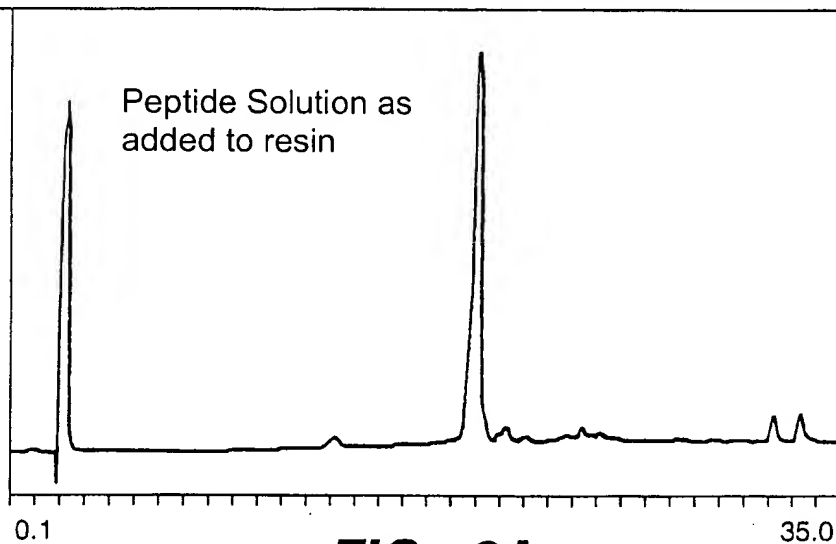


FIG._8A

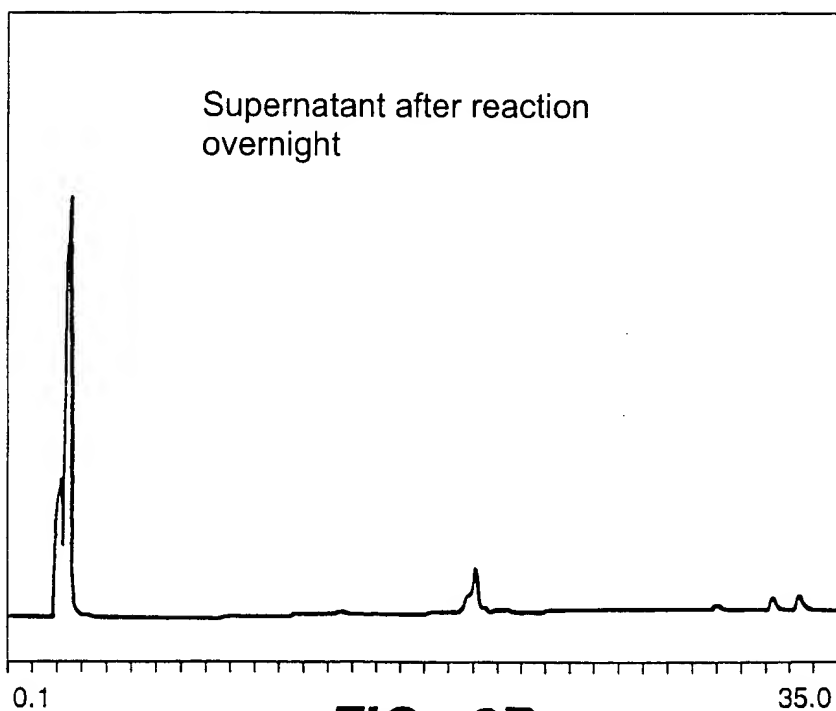


FIG._8B

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH(1)
+ Resin - PCL - ONH₂

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH(1)
MALDI MASS = 4022, BASE CLEAVAGE MASS = 3745

Polymer-
Supported
Ligation on
ISCO

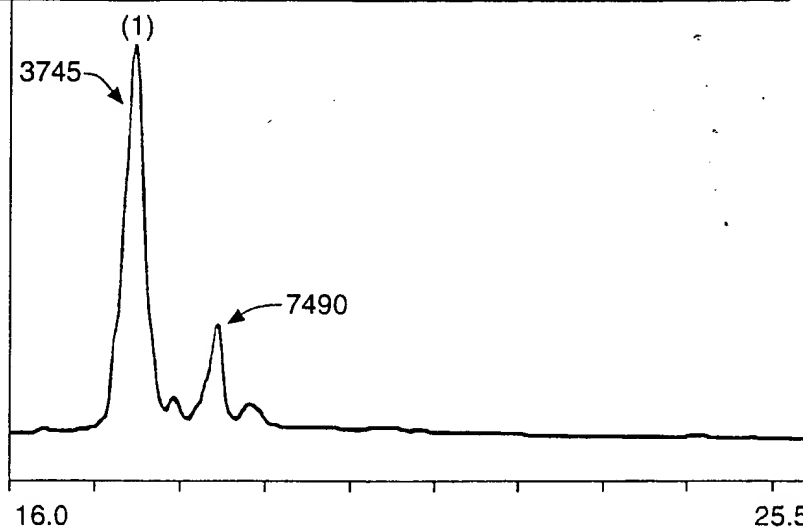


FIG._9A

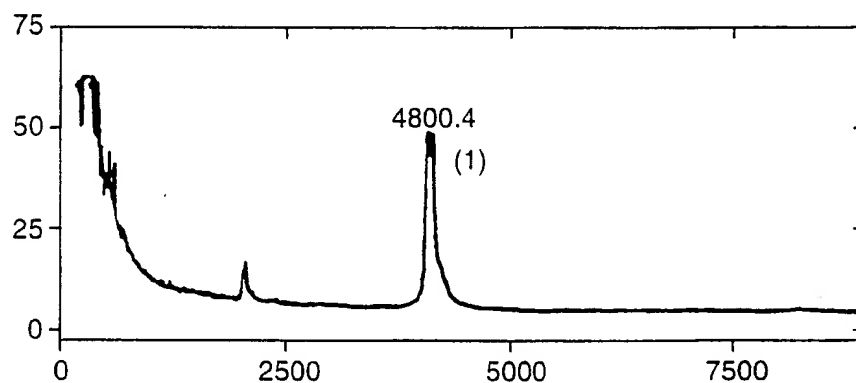


FIG._9B

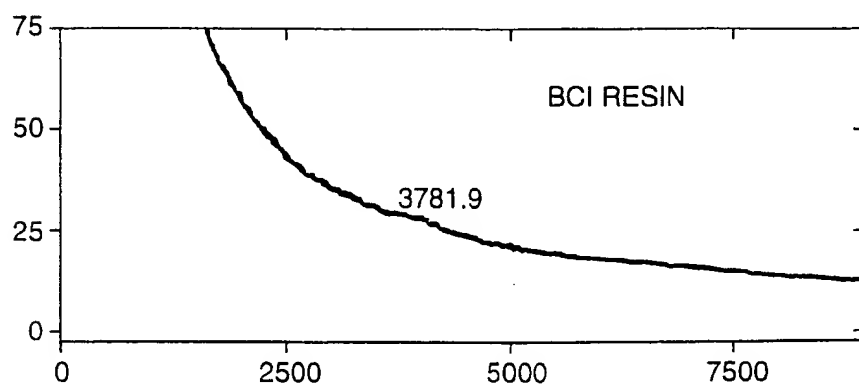


FIG._9C

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Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSAc (1)
MALDI MASS = 4080, BASE CLEAVAGE MASS = 3729
+ H - CGFRVREFGDNTA - COSH (2)
↓ 3. pH 7.5, 6M Gu•HCl, 0.1M PHOSPHATE, 0.5% THIOPHENOL
Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHGCGFRVREF -
GDNTA - COSH (1+2)
MALDI MASS = 5476, BASE CLEAVAGE MASS = 5199

Polymer-Supported
Ligation on
ISCO

FIG._10A

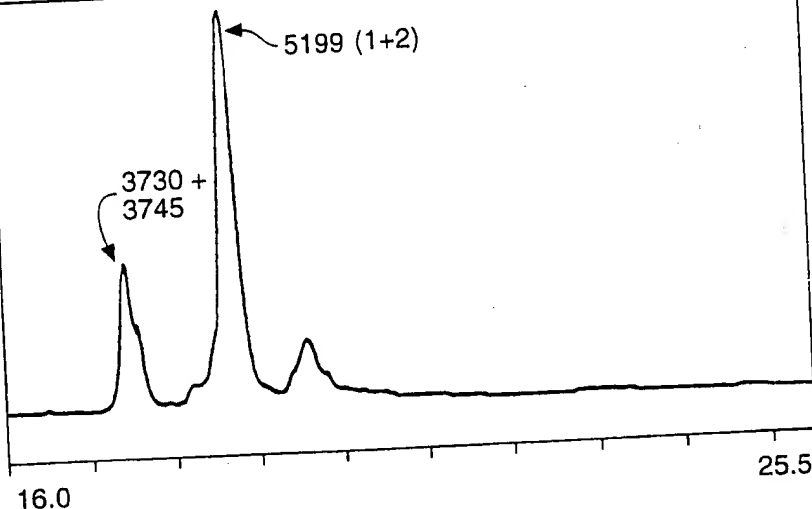


FIG._10B

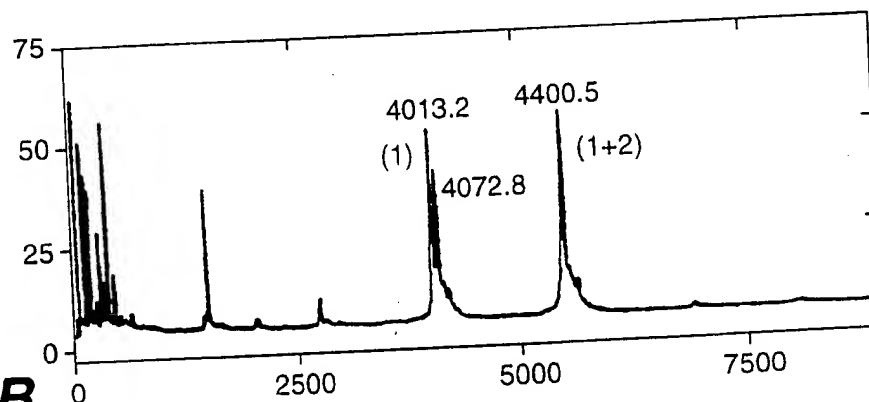
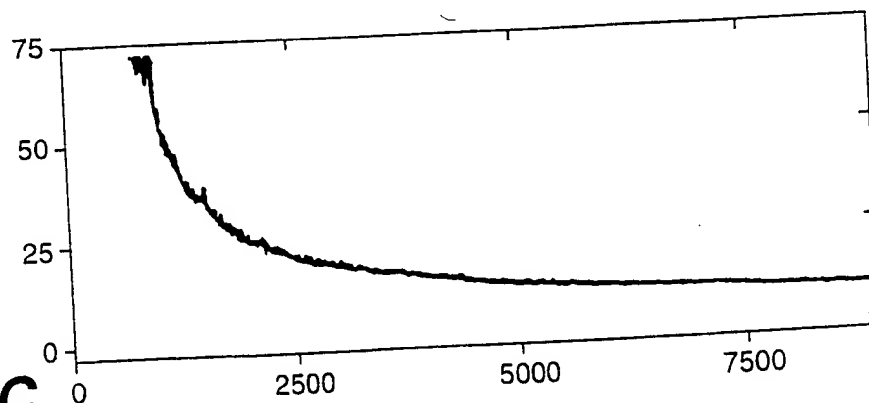


FIG._10C



Polymer-Supported
Ligation on ISCO

FIG. 11

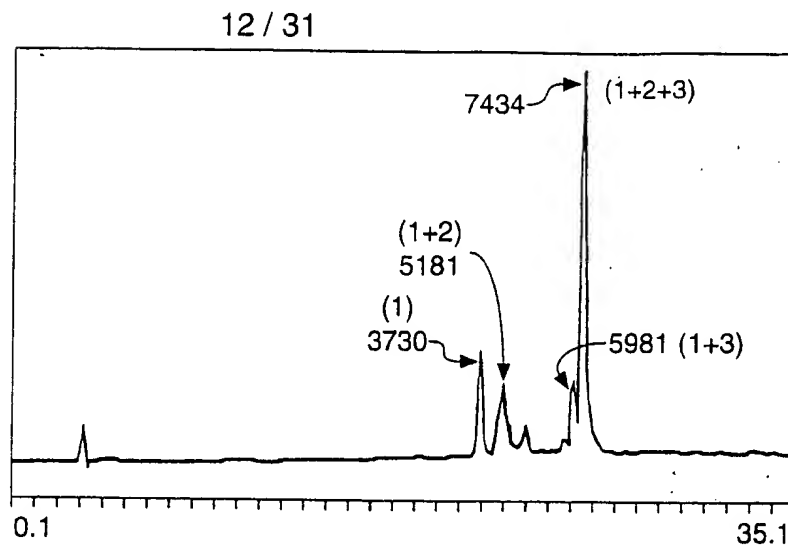


FIG. 12A

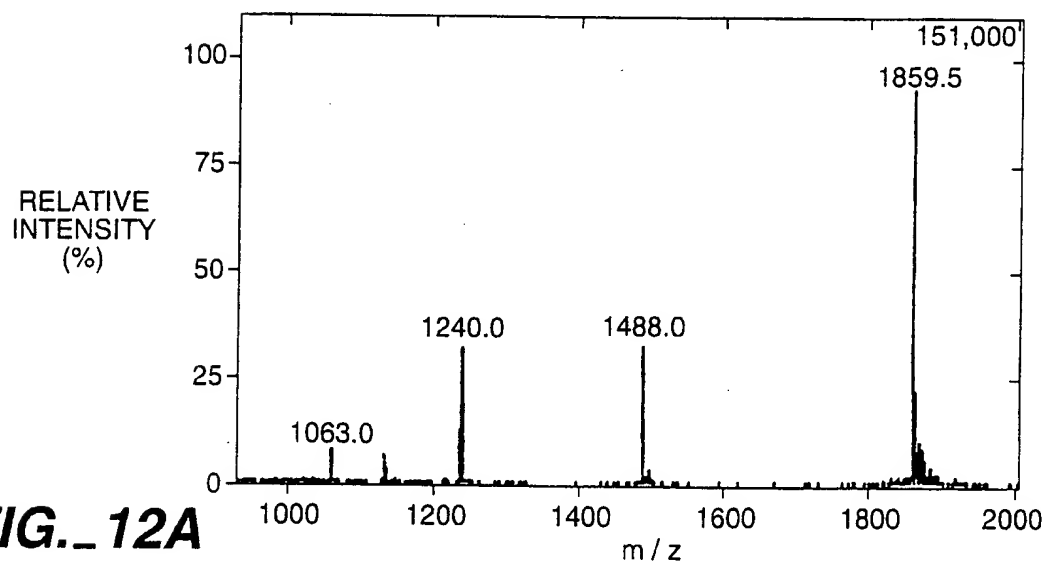
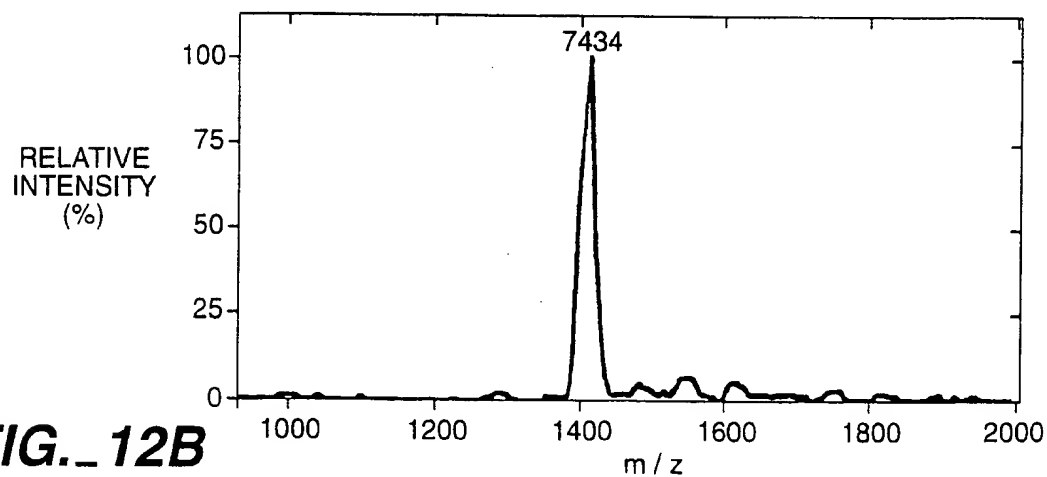
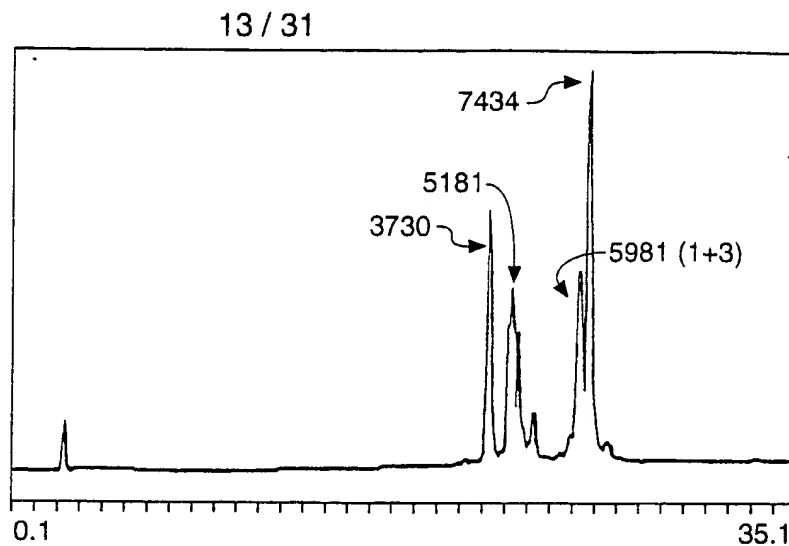


FIG. 12B



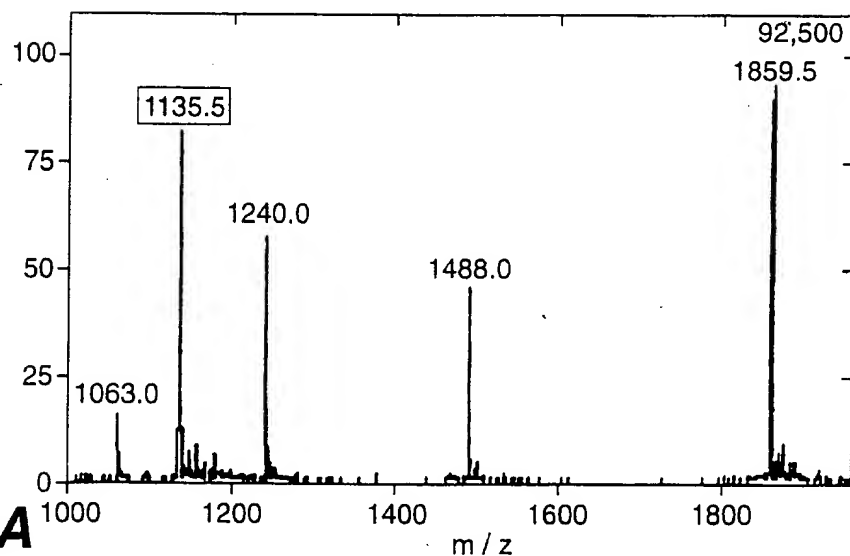
Polymer-Supported
Ligation on
PEGA

FIG._ 13



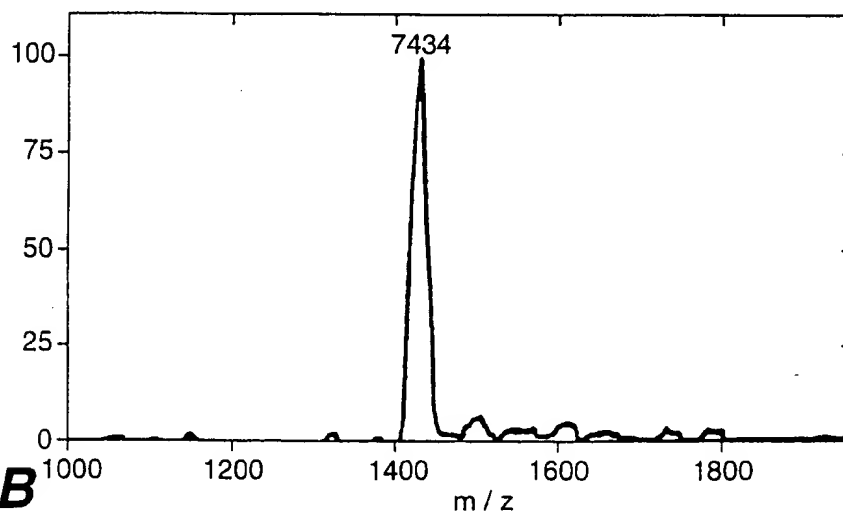
RELATIVE
INTENSITY
(%)

FIG._ 14A



RELATIVE
INTENSITY
(%)

FIG._ 14B



On Resin
Purification

FIG._ 15A

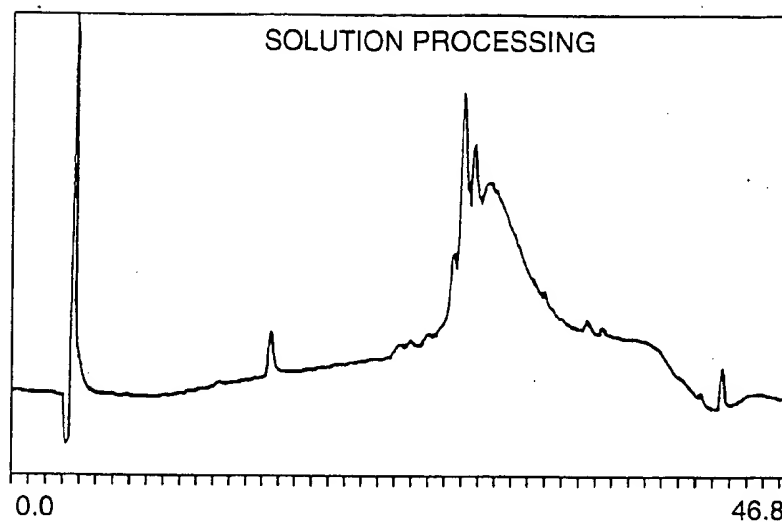


FIG._ 15B

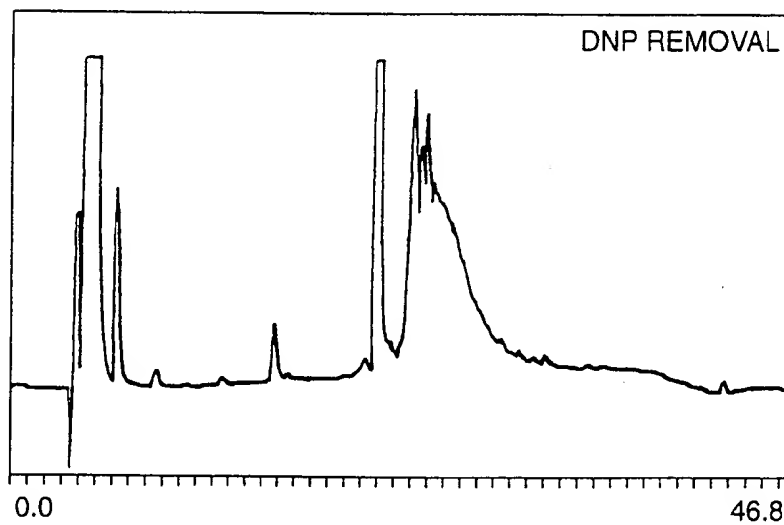
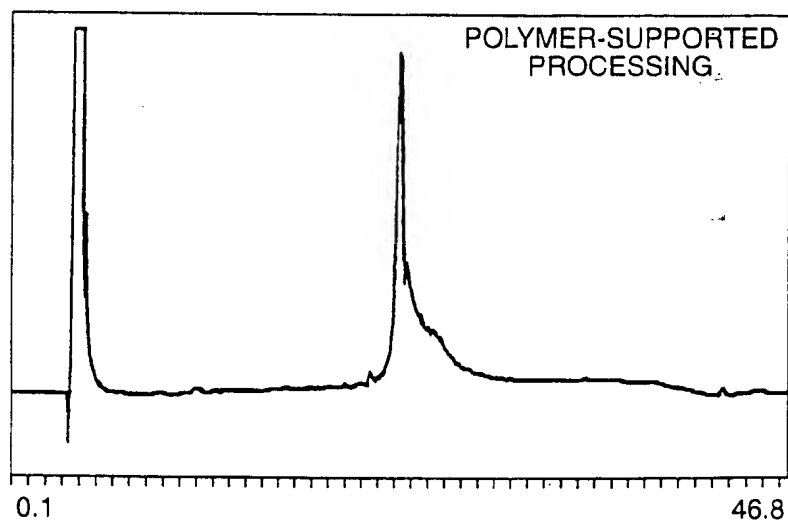
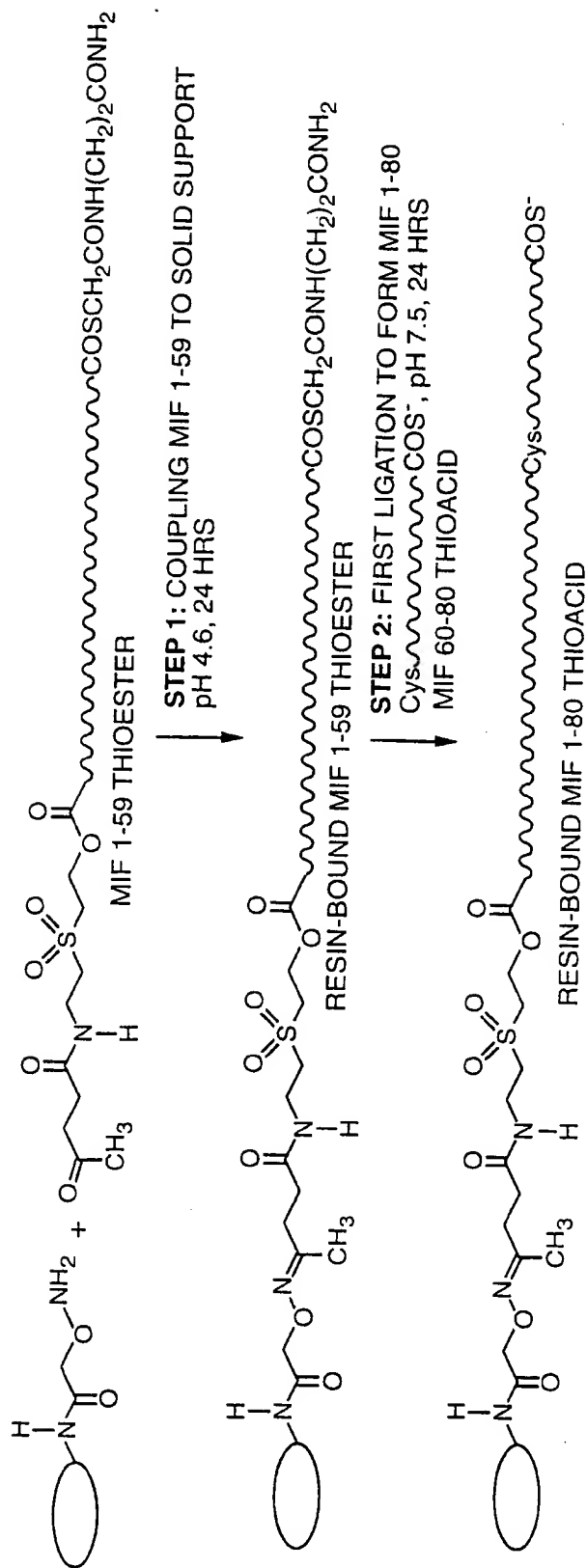


FIG._ 15C



**FIG. 16A**

Synthesis of MIF by Solid Phase Native Ligations

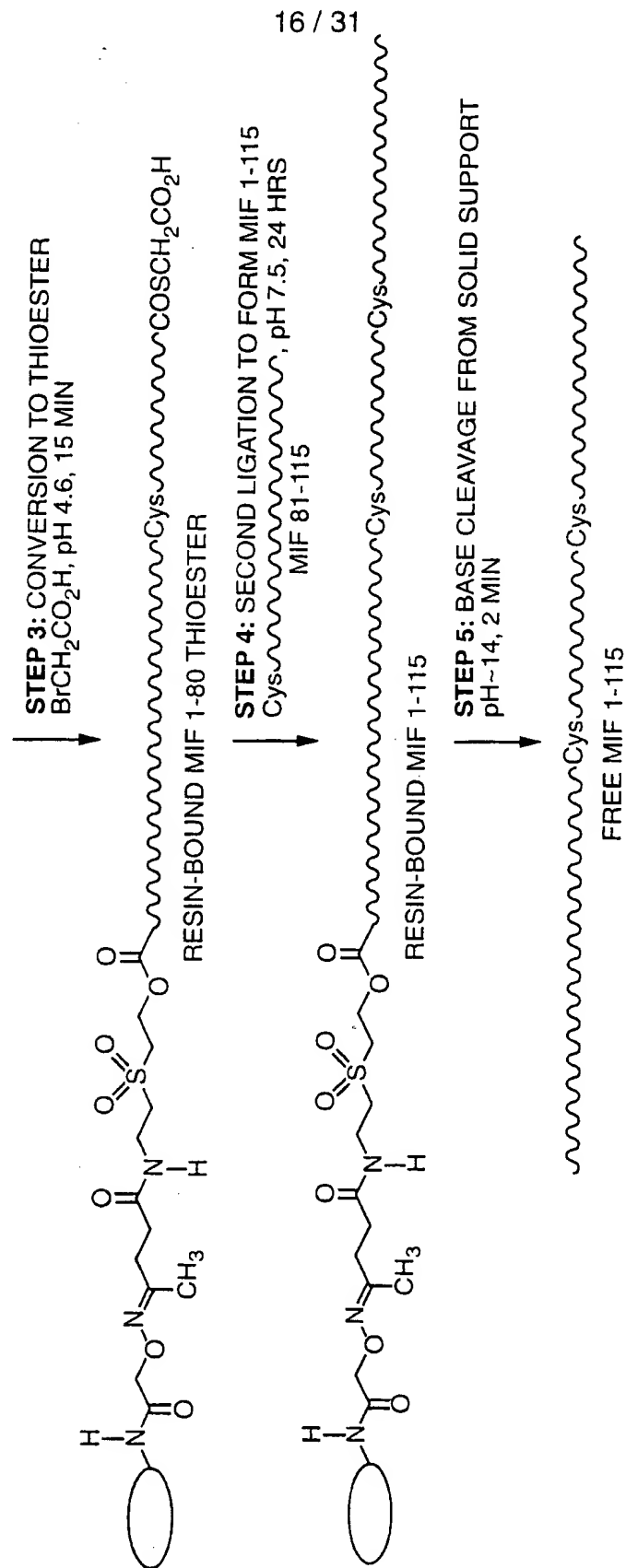
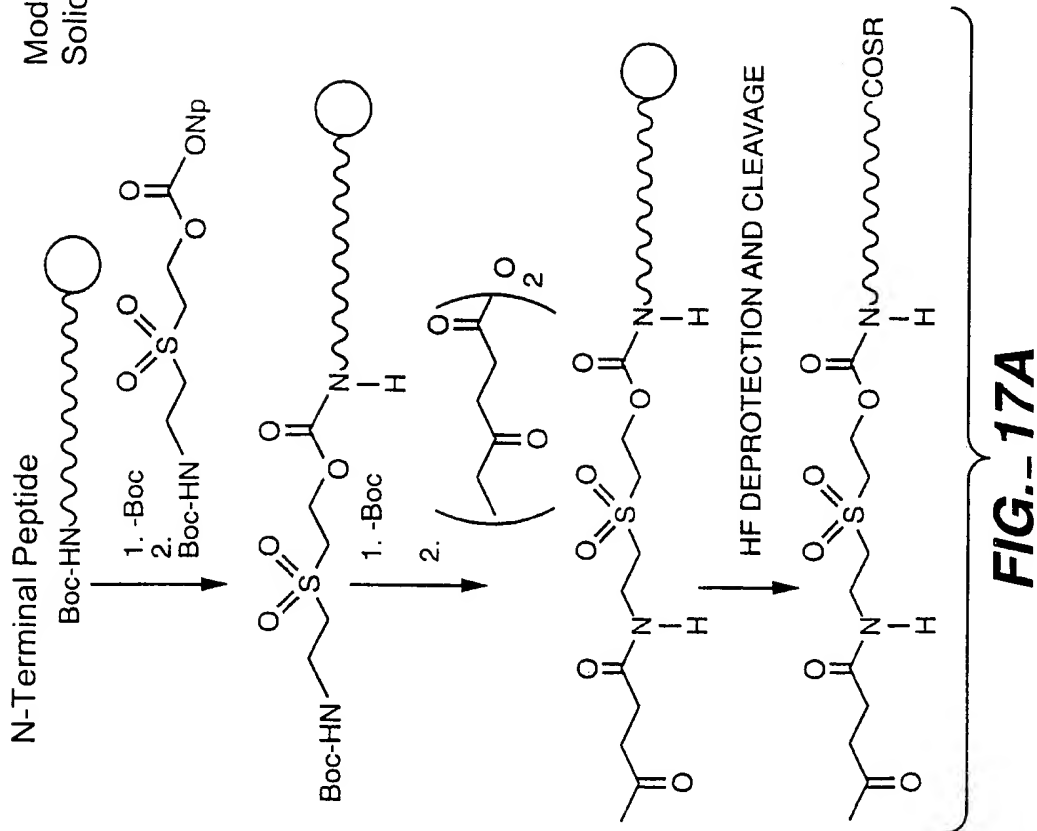
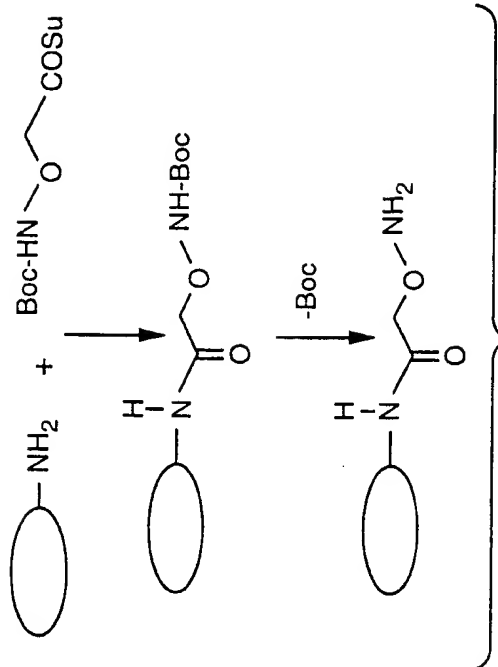


FIG. 16B

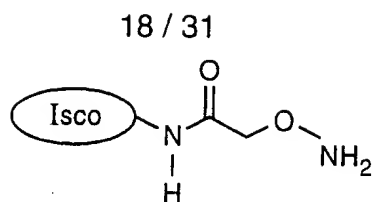
Modification of N-Terminal Peptide Segment and
Solid Support



Solid Support



Coupling of MIF
1-59 to Solid
Support



KETONE - MSC HANDLE - MET¹ - MIF 2 - 58 - Leu⁵⁹ - SAc - βAla - CO₂H

#1

+



Isco - OXIME - MSC HANDLE - MET¹ - MIF 2 - 58 - Leu⁵⁹ - SAc - βAla - CO₂H
EXPECTED BASE CLEAVAGE MASS = 6271

FIG._18A

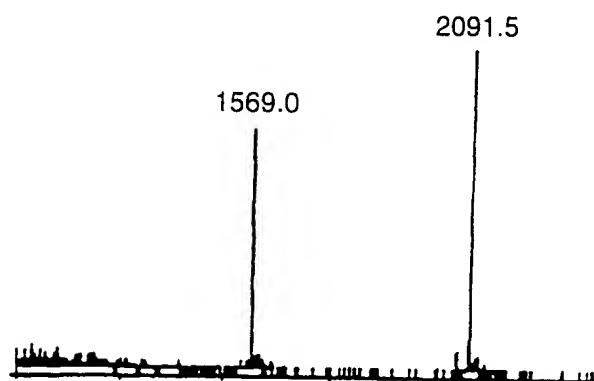


FIG._18C

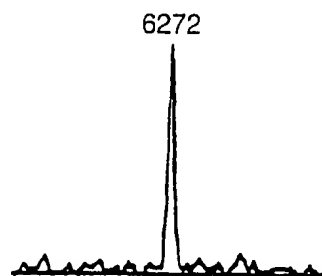
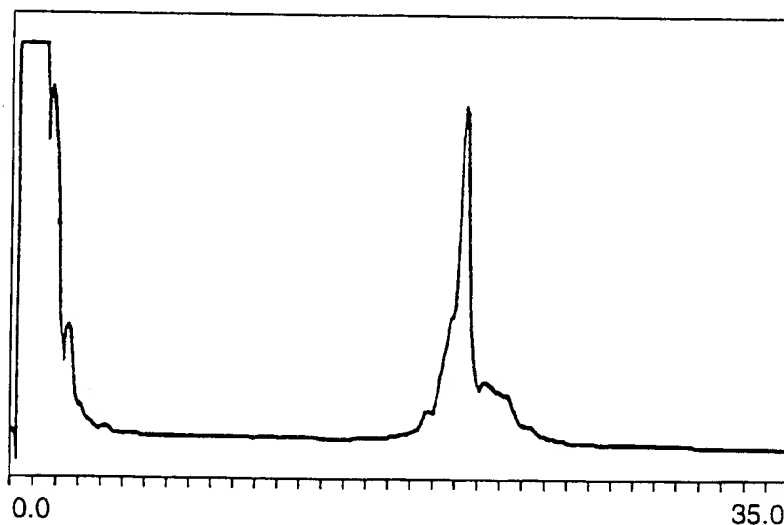
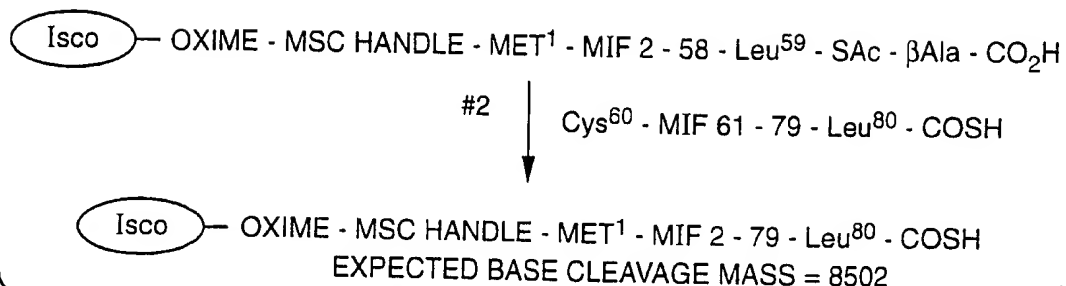
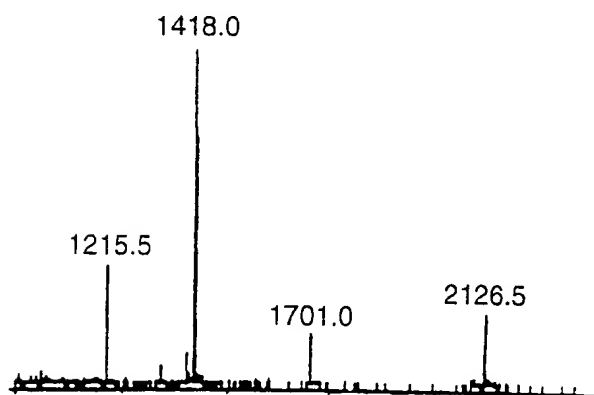
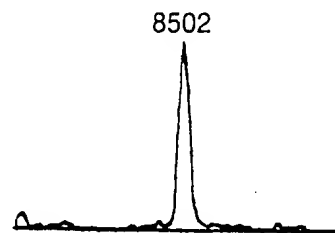
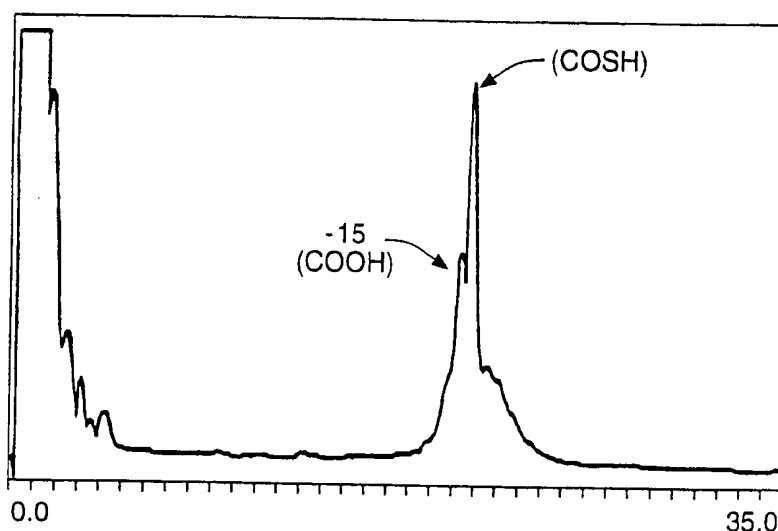


FIG._18D

FIG._18B



Ligation to form MIF 1-80

**FIG. 19A****FIG. 19C****FIG. 19D****FIG. 19B**

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Ligation to form MIF 1-115

Isco — OXIME - MSC HANDLE - MET¹ - MIF 2 - 79 - Leu⁸⁰ - COSAc

#4 ↓ Cys⁸¹ - MIF 82 - 114 - Ala¹¹⁵ - CO₂H
6M Gu•HCl, 0.1, 0.1 M Na Pi, 0.5% THIOPHENOL
↓ 0.15 M METHIONINE, pH 7.5

Isco — OXIME - MSC HANDLE - MET¹ - MIF 2 - 114 - Ala¹¹⁵ - CO₂H
EXPECTED BASE CLEAVAGE MASS = 12450

FIG._20A

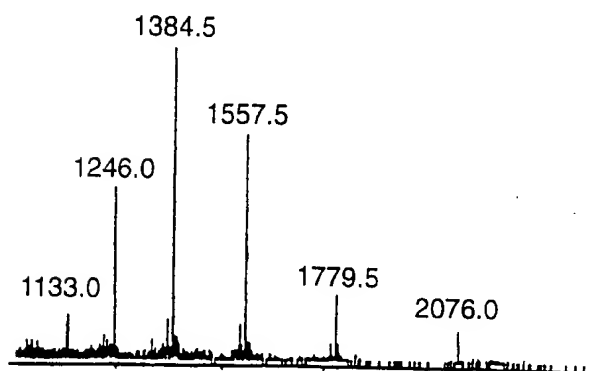


FIG._20C

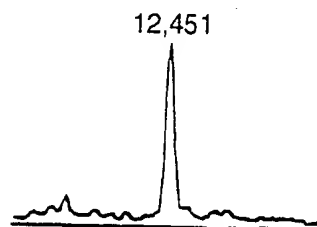


FIG._20D

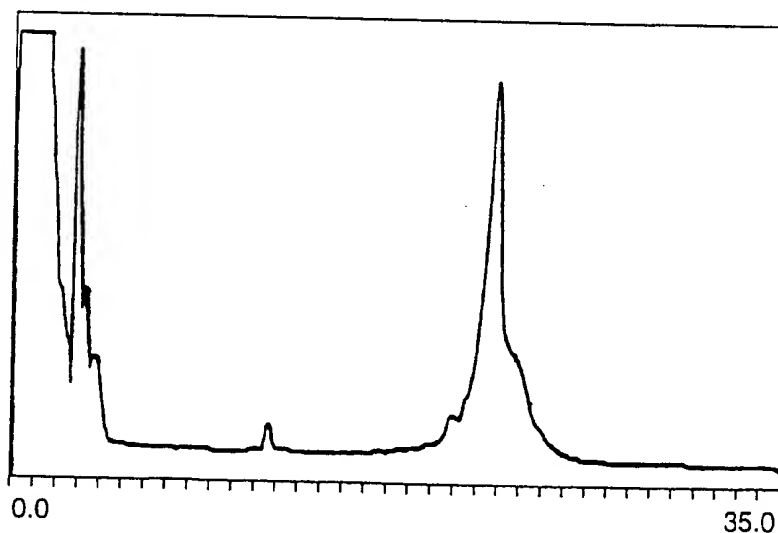


FIG._20B

Solid Phase Chemical Ligations in the
C- to N-terminal Direction

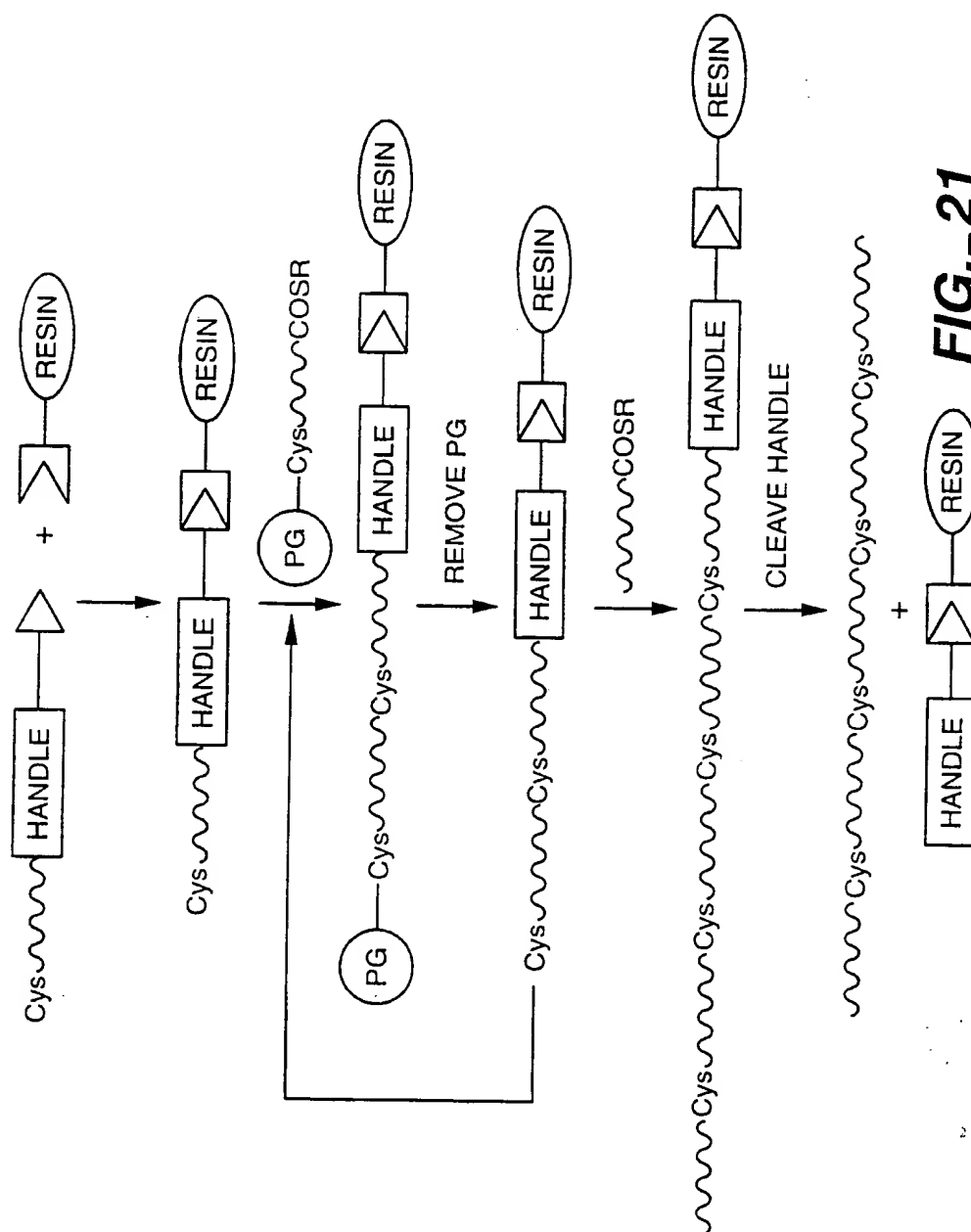


FIG. 21

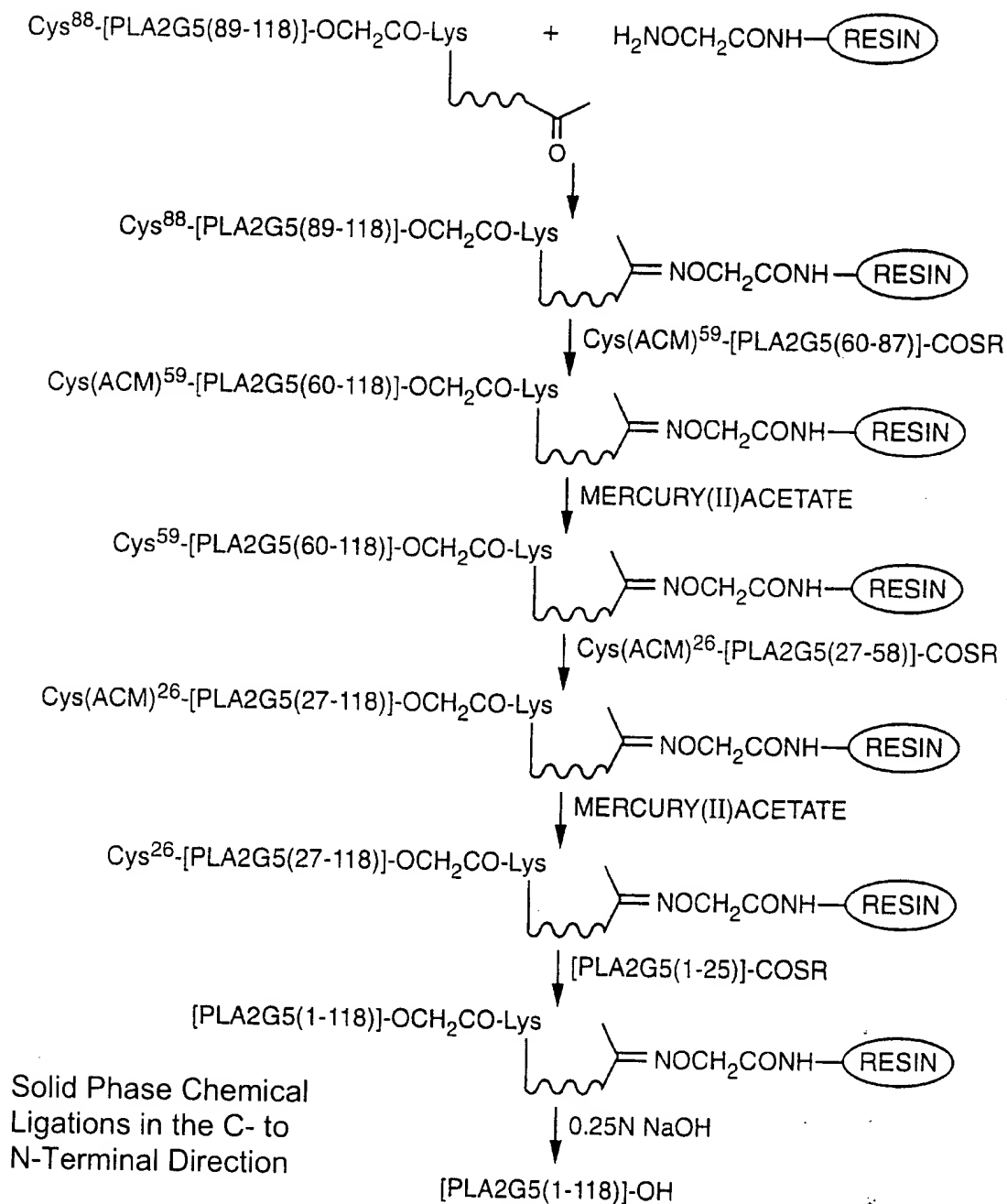
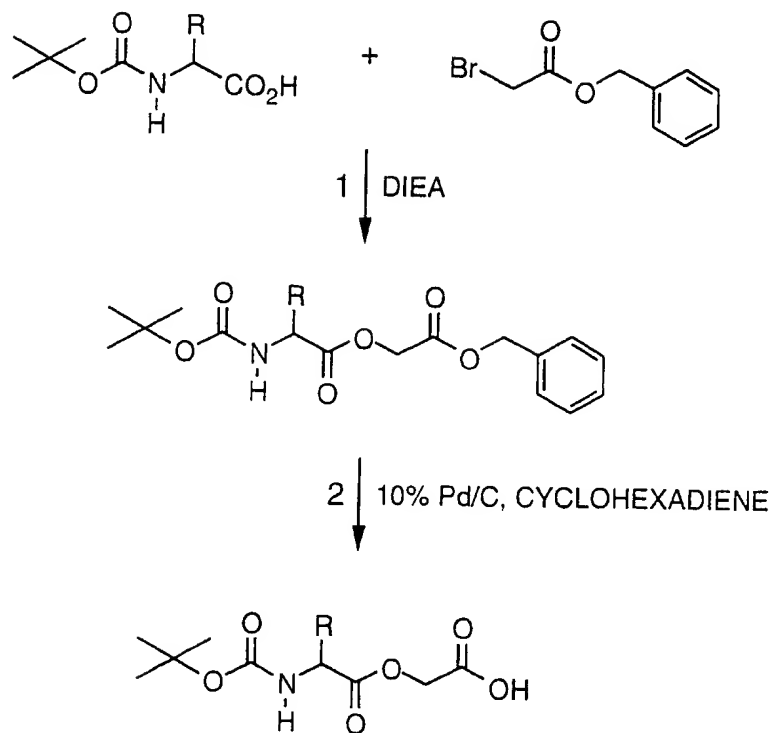
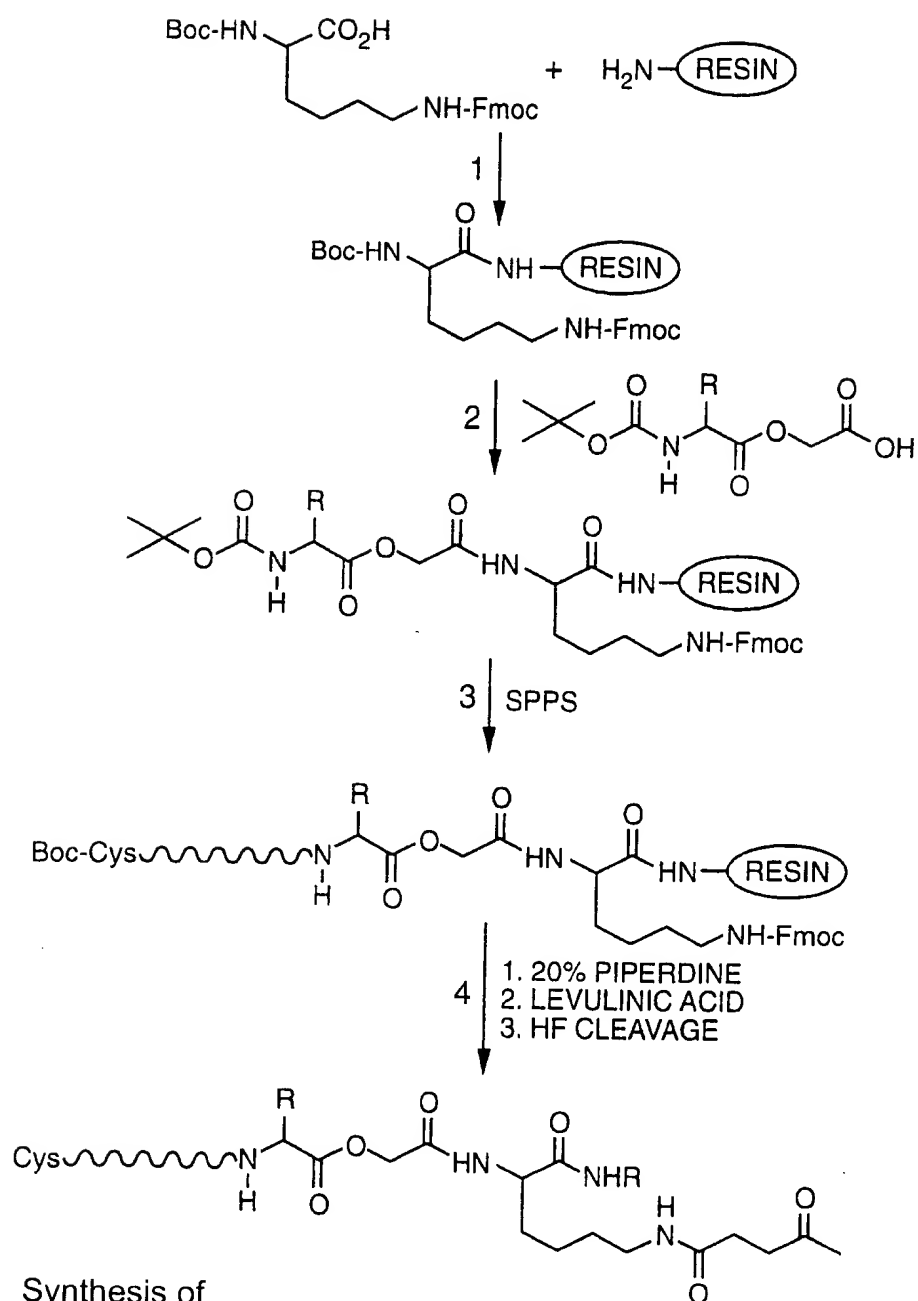


FIG. 22

**FIG. 23**

Synthesis of Cam ester derivative



Synthesis of
C-Terminal Peptide
Segment

FIG. 24

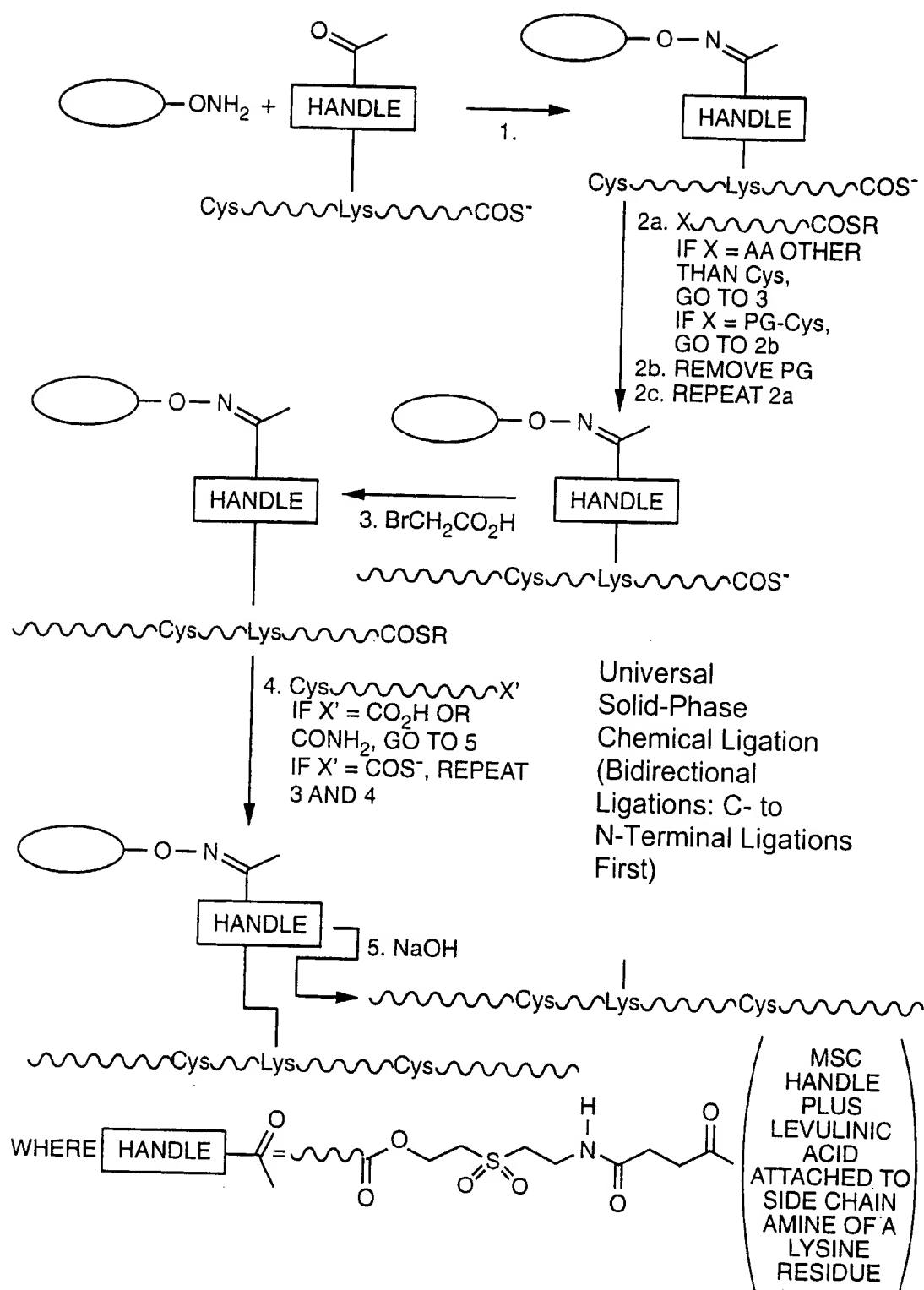


FIG. 25A

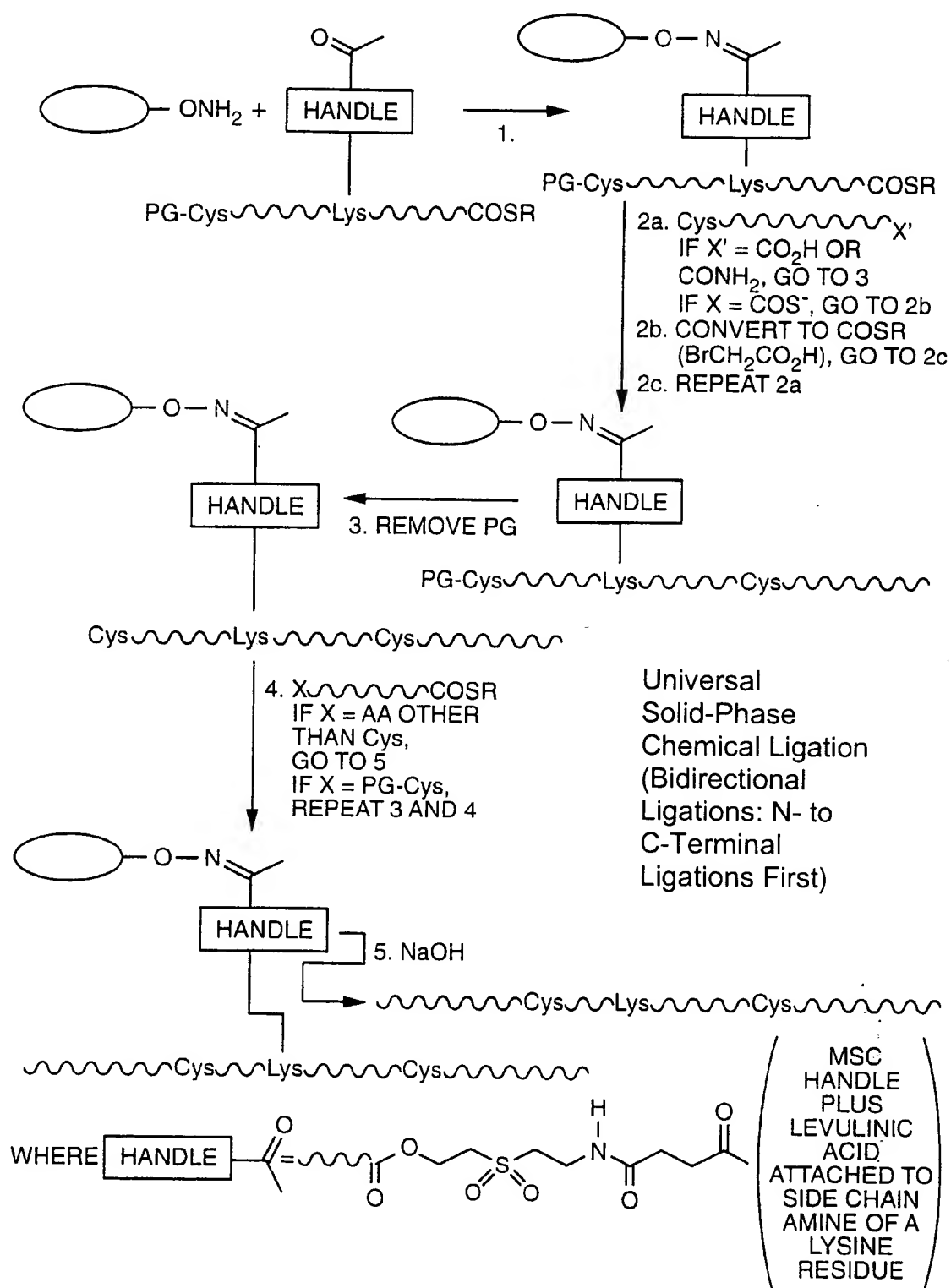

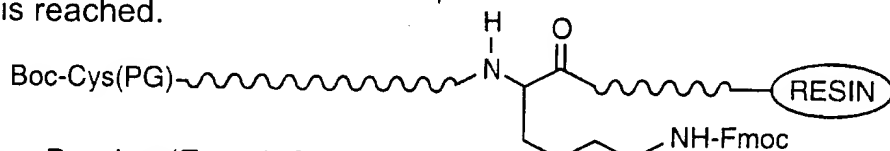


FIG. 25B

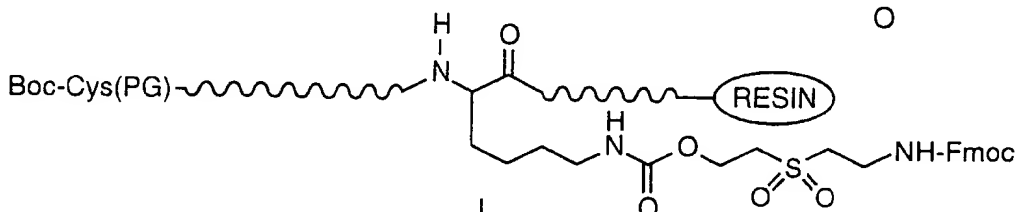
Boc-Cys(PG)~~~~~(RESIN)

1. Boc
2. Boc-HN 
3. -Boc
4. SYNTHESIS OF REST OF SEGMENT

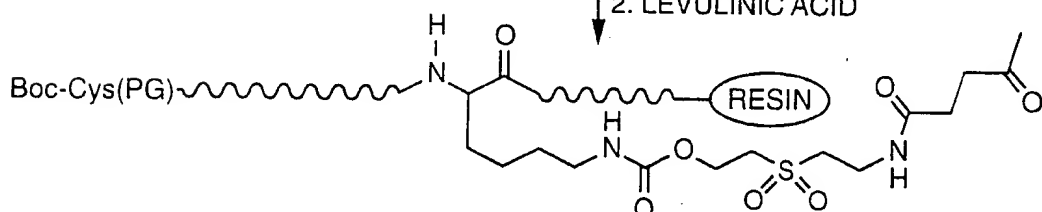


Couple a Boc-Lys(Fmoc)-OH, then continue the rest of the synthesis

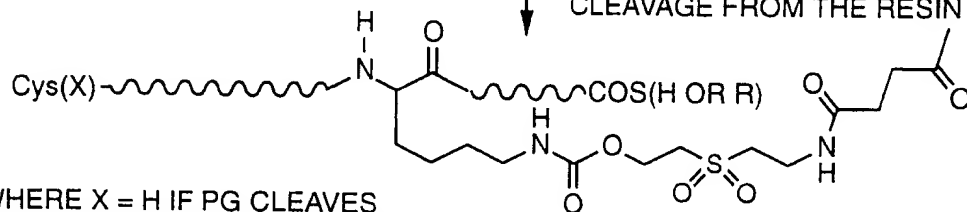
1. -Fmoc (1-2 eq OF DBU IN DMF)
2. Fmoc-HN-CH₂-CH₂-S(=O)₂-CH₂-CH₂-O-C(=O)-OR



1. -Fmoc (1-2 eq OF DBU IN DMF)
2. LEVULINIC ACID

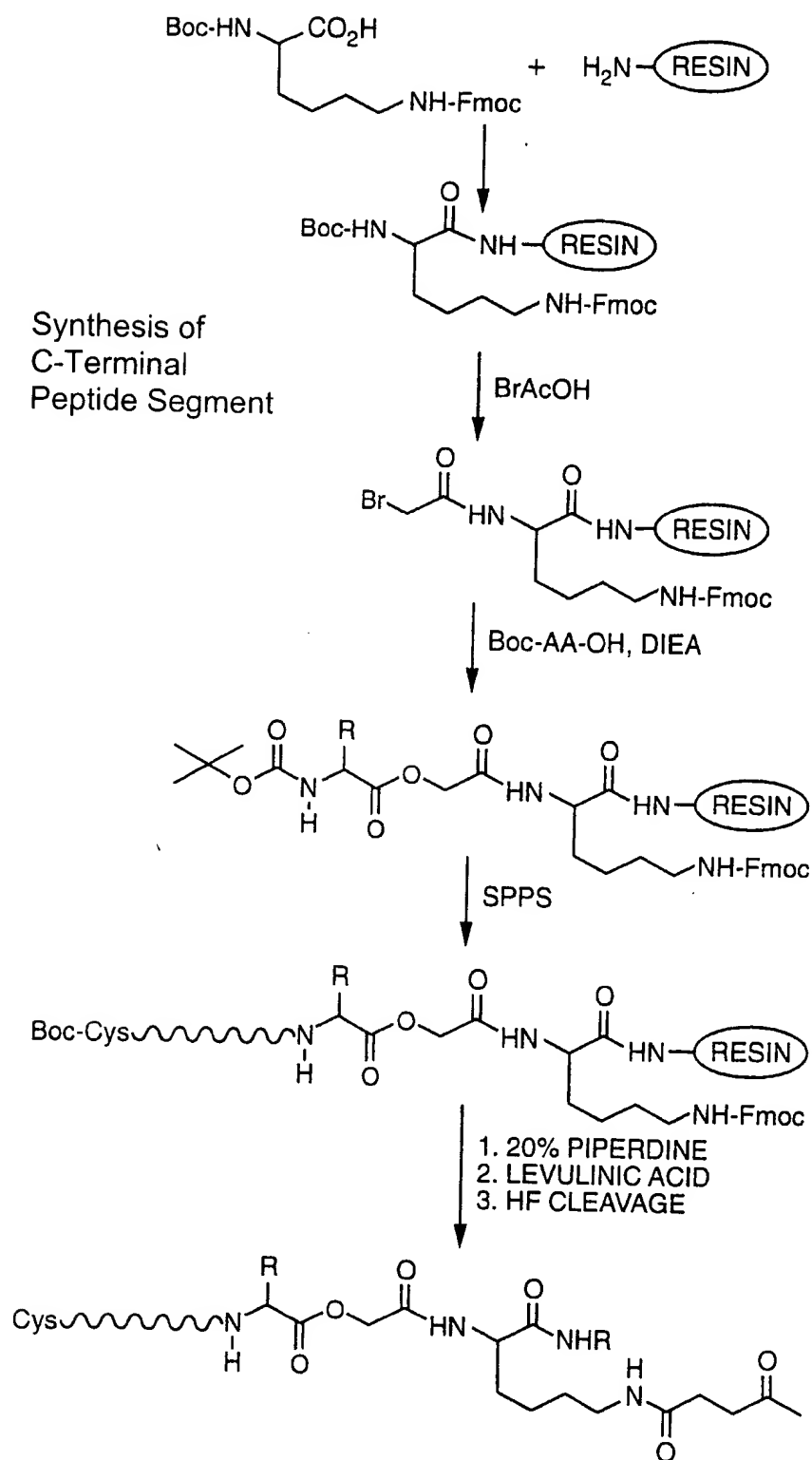


1. -Boc
2. HF DEPROTECTION AND CLEAVAGE FROM THE RESIN



WHERE X = H IF PG CLEAVES
IN HF AND WHERE X REMAINS
PG IF PG IS STABLE TO HF

FIG. 25C

**FIG. 27**

ALTKYGFYGCYGRLEEKGCADRKNILA

1 10 19 27

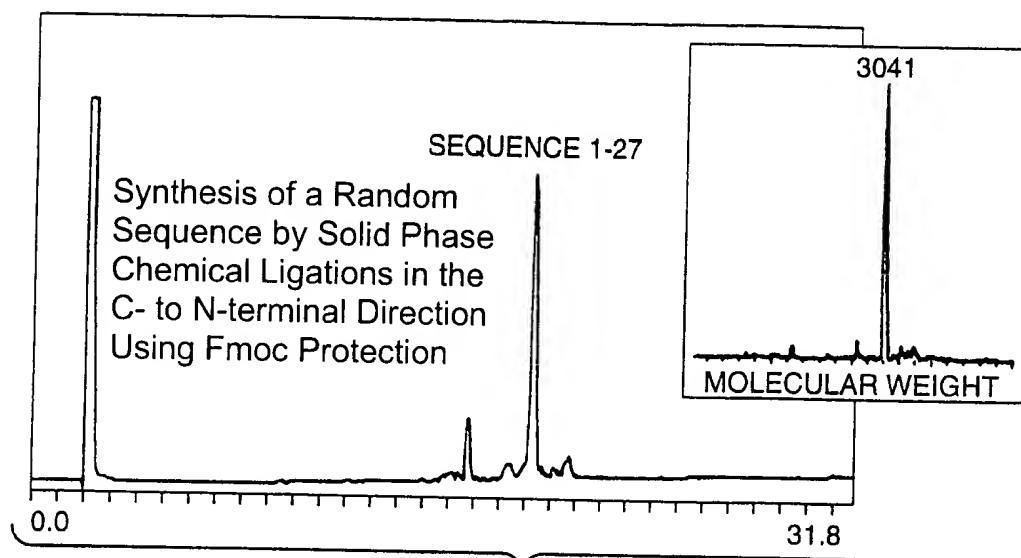


FIG._28

ALTKYGFYGCYGRLEEKGCADRKNILA

1 10 19 27

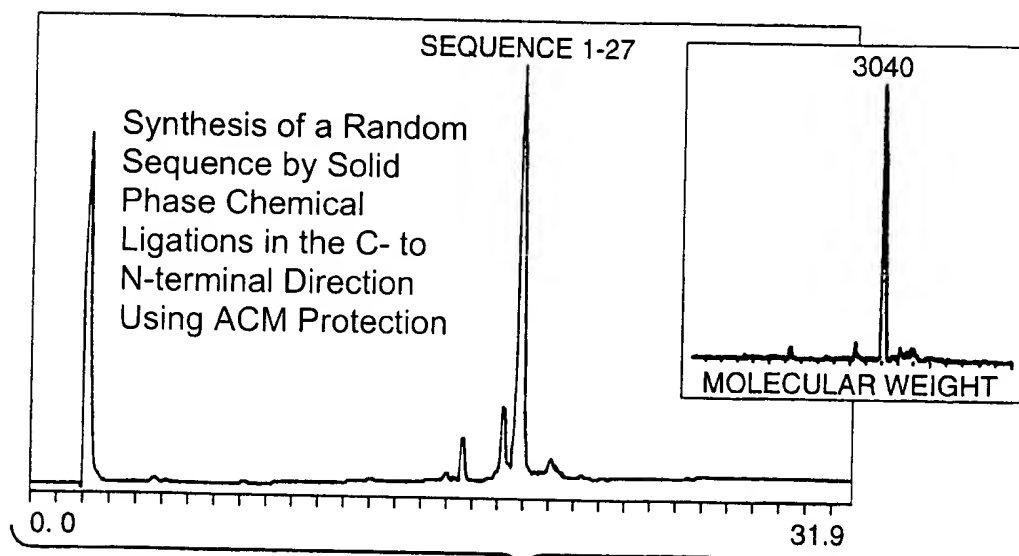
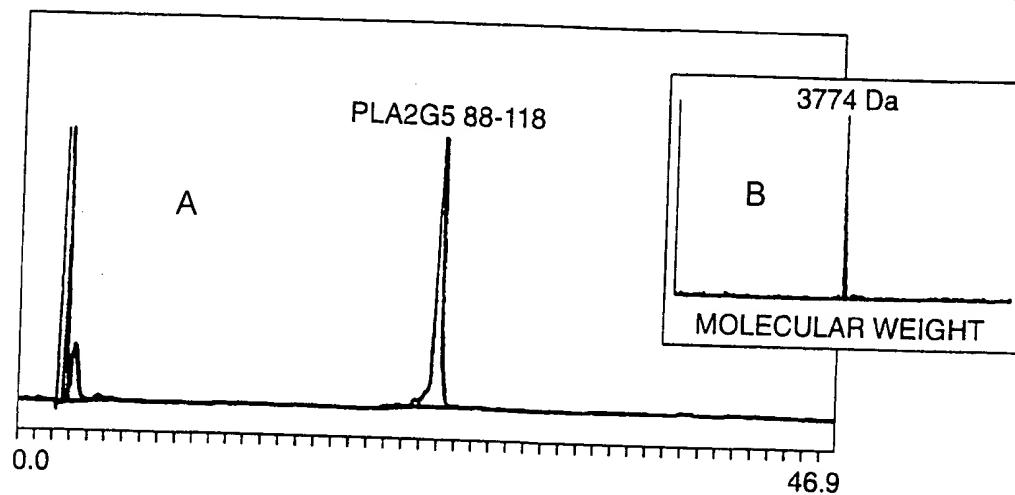


FIG._29

1 26 59
GLLDLKSMIEKVTGKNALTNYGFYGCYCGWGGRGTPKDGTWCCWAHDHCYGRLEEKGC
NIRTQSYKYRFAWGVVTCEPGPFCHVNLCA⁸⁸CDRKL¹¹⁸VYCLKRN¹¹⁸LSYNPQYQYFPN¹¹⁸ILCS



1 26 59
GLLDLKSMIEKVTGKNALTNYGFYGCYCGWGGRGTPKDGTWCCWAHDHCYGRLEEKGC
NIRTQSYKYRFAWGVVTCEPGPFCHVNLCA⁸⁸CDRKL¹¹⁸VYCLKRN¹¹⁸LSYNPQYQYFPN¹¹⁸ILCS

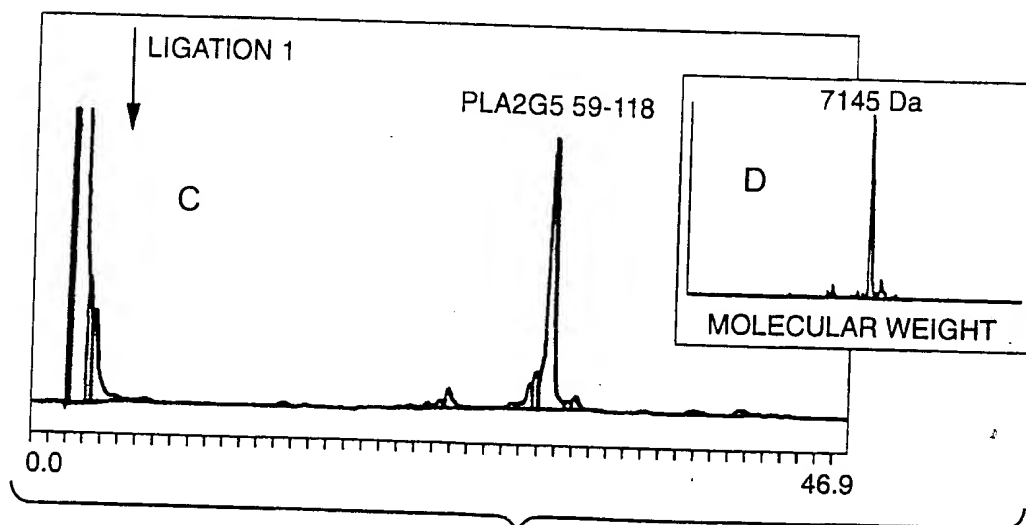


FIG. 30

FIG. 30

